AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

METHOD DEVELOPMENT AND VALIDATION OF CLOBAZAM IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY USING SPECTROPHOTOMETRIC METHOD

Parag. S. Mahadik*[1], Senthilkumar. G. P[1], Devprakash Dahia[1], T. Tamizh Mani[1], Priyanka. K. Gaikwad[2].

1. Bharathi college of Pharmacy, Bharathinagara.
2. Tatyasaheb kore college of pharmacy, Warananagar.

ABSTRACT

In the present research a simple, accurate, precise and cost effective UV-Vis spectrophotometric method for the estimation of Clobazam, in bulk and pharmaceutical dosage form was illustrated. The absorption maxima of the drug was found to be 230 nm in methanol: water (1:1). A linear response was observed in the range of 6- 16 µg/ml with a regression coefficient of 0.999. Validation parameters were carried out as per the guidelines of International Conference for Harmonization. This method can be used in the industries for determination of Clobazam to analyze the quality of formulation without interference of the excipients.

Key words: Clobazam, Anti-epileptic, λmax, ICH, UV-Vis spectroscopy.

INTRODUCTION

Clobazam is an antiepileptic drug belonging to the vast benzodiazepine (Librium) series coming under the class of Anticonvulsant drugs[1]. Benzodiazepines have gained popularity over barbiturates as hypnotics and sedatives. The reference of Clobazam is not found in majority of pharmaceutical and chemistry books. Clobazam is found to be official in Indian Pharmacopia 2007. Today majority of marketed antiepileptic dosage forms are of Clobazam *eg*. Frisium, Urbanyl, *etc*, After doing the thorough literature review it was found that analytical methods for

*Corresponding Author Email: drsenn78@yahoo.com

Received 28 October 2011, Accepted 10 November 2011

Please cite this article in press as: Mahadik PS *et al.*, Method Development and Validation of Clobazam in Bulk and Pharmaceutical Dosage Forms by Using Spectrophotometric Method. American Journal of PharmTech Research 2011.

estimation of Clobazam in bulk and in dosage form are not available in majority. There are several research paper which illustrates the method for estimation of Clobazam by colorimetry and HPLC in bulk and pharmaceutical dosage form[2,3]. There have been several bio-analytical methods developed for Clobazam in biological fluids containing Clobazam, like serum and plasma. But, there have been very less number of analytical methods developed for estimation of Clobazam in pure bulk form and in dosage form. The structure of Clobazam contains the conjugated double bonds. It also contains functional groups like chlorine, two carbonyl groups and nitrogen. So there is a scope for development of methods like UV spectroscopy and Colorimetry[2, 4, 5]. Further serum Clobazam was also monitored using isothermal gas-liquid chromatography by using nitrogen detector[6]. In the present study method development and validation was carried out by using spectroscopic method[7,8].

MATERIAL AND METHOD

Analytical grade methanol was used as a solvent for dilution. And water used for dilution was distilled in the laboratory. A double beam UV spectrophotometer (Shimadzu UV-1800) was used with 1 cm matched quartz cell. Tablet formulation [Frisium, Sanofi-aventis Ltd., Goa, India and Cloba-5, Intas pharmaceuticals Ltd., Ahmedabad, India] were procured from a local pharmacy with labeled amount 5 mg per tablet.

Zero Order Spectroscopic Method and Area Under Curve Method

Solvent selection

Various solvents were selected for the solubility studies and it was found that Clobazam was soluble in the following solvents; dimethyl sulfoxide, dimethyl formamide, methanol, chloroform, acetonitrile, *etc*. In the present investigation methanol: water (1:1) was selected as a solvent.

Selection of analytical wavelength and absorption maxima

Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm.

Preparation of stock solutions

Standard Clobazam 50 mg was weighed and transformed to a 50 ml volumetric flask and dissolved in 25 ml of methanol. The flask was shaken and volume was made up to the mark with water to give a solution containing 1000 µg/ml (Stock solution A). From this stock solution A, pipette out 10 ml and place into 100 ml volumetric flask. The volume was made up to the mark with methanol: water (1:1) to give a solution containing 100 µg/ml (Stock solution B).

Selection of analytical concentration range

From the standard stock solution B of Clobazam, appropriate aliquots 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml were pipetted out in 10 ml volumetric flasks and dilutions were made with methanol: water (1:1) to obtain working standard solutions of concentrations from 6-16 µg/ml. Absorbance for these solutions were measured at 230 nm. For standard solution analytical concentration range was found to be 6-16 µg/ml and overlain spectra was obtained.

Sample preparation for determination of Clobazam from Dosage form

Twenty tablets of each formulation [Frisium, Sanofi-aventis Ltd., Goa, India, Intas pharmaceuticals Ltd., Ahmedabad, India] were weighed and finely powdered. The powder equivalent to 50 mg of Clobazam was accurately weighed and transferred to volumetric flask of 50 ml capacity containing 25 ml of methanol and was sonicated for 30 min. The flask was shaken and the solution was filtered through Whatmann filter paper (No. 41) into 50 ml volumetric flask. To the volumetric flask, 25 ml of distilled water was added and volume was made up to the mark with methanol to give a solution of 1000 µg/ml (Stock solution A). From this solution 10 ml was taken and placed in 100 ml volumetric flask. The volume was made up to the mark using methanol: water (1:1) to give a solution of 100 µg/ml (Stock solution B). From the stock solution B, 8.0 ml was taken and diluted to 10 ml to give 8 µg/ml and it was further used for the estimation of Clobazam.

METHOD VALIDATION

The method was validated as per International Conference on Harmonization (ICH) guidelines

Linearity

Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyst. Linearity was evaluated by visual inspection of a calibration graph. At least three concentration levels were tested in agreement to ICH. The slope, intercept was reported as required by ICH. LOD and LOQ were estimated from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the intercepts of the regression lines done in the range of the detection limit.

Accuracy

The accuracy of the method was measured by recovery studies and ascertained by standard addition method. A known amount of pure drug at three different levels i.e. 80 %, 100 %, and

120 % was added to pre-analyzed sample solutions and total concentration was determined using the proposed method.

Precision

Precision was investigated at three levels, intra-day, inter-day, and reproducibility. The intra- and inter- day variability were assessed by using standard drug solution at three different concentration. Intra-day precision was carried out by analyzing the drug solutions within same day. The inter-day precision was measured using standard solution over three consecutive days. Reproducibility of the method was determined by performing same analytical procedure at different laboratories using same experimental design.

Limit of Detection & Limit of Quantitation:

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, though not necessarily quantitate. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

RESULTS AND DISCUSSION

Selection of analytical wavelength and absorption maxima (R)

The absorption spectra thus obtained was derivatized for zero order spectroscopy. This zero order spectrum was selected for the analysis of the drugs. The absorption maximum was found at 230 nm which can be further used for analysis and for area under curve the absorption was found to be between 225 and 235. (Figure 1 and 2).

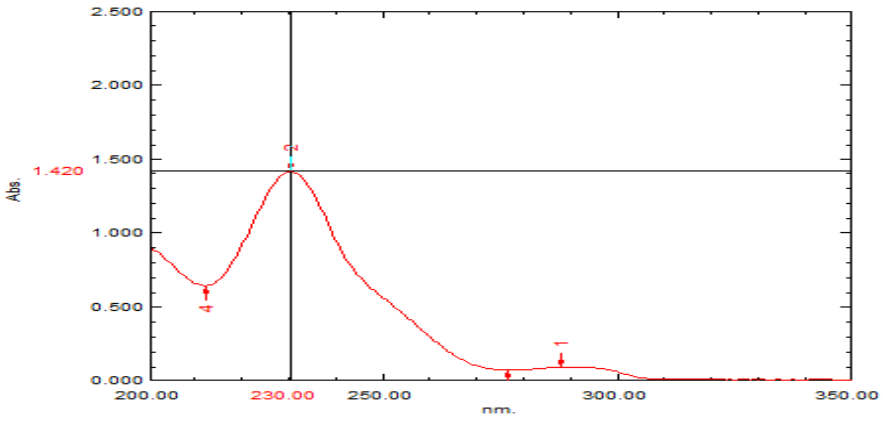

Figure 1.UV spectrum of Clobazam standard by zero order method

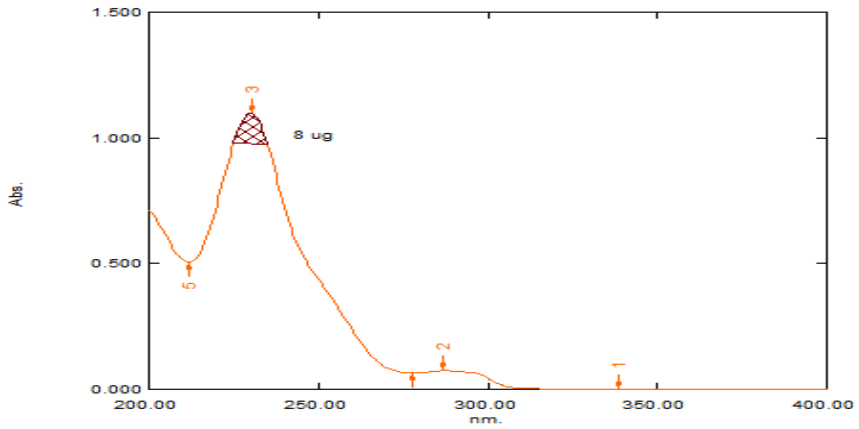

Figure 2. Area under curve spectra of Cobazam standard

Calibration curve for the Clobazam

Appropriate volumes of aliquots from standard Clobazam stock solution B were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol: water (1:1) to obtain concentrations of 6, 8, 10, 12, 14 and 16 µg/ml. Absorbance value of each solution against methanol: water (1:1) as a blank were measured at 230 nm. From that absorbance value, regression equation and correlation coefficient (r^2) were determined and presented (Table 1 and 2).

Table. 1. Absorbance values for calibration curve of Clobazam at 230 nm by zero order spectroscopy

Concentration (µg/ml)	Absorbance*	±S.D*
0	0	±0
6	0.834667	±0.000577
8	1.098333	±0.000577
10	1.416	±0.001
12	1.673667	±0.002082
14	2.002667	±0.001528
16	2.273333	±0.005132
	Average of SD	**±0.001816**

*is the average of three determinations.

VALIDATION PARAMETERS

Linearity

The linear regression equation and the statistical evaluation of the calibration plots for the analysis of authentic samples are listed in Table 3. Under the described experimental conditions, linear correlations were obtained at the wavelength 230 nm over the concentration range of 6-16

Table. 2. Area under curve values for calibration curve of Clobazam at 225-235 nm

Concentration (µg/ml)	Average Area under curve*	±S.D*
6	**0.605667**	±0.000577
8	**0.787**	±0.002646
10	**1.023**	±0.003606
12	**1.21**	±0.004359
14	**1.456667**	±0.015535
16	**1.623333**	±0.011504
	Avg S.D=	**±0.006371**

*is the average of three determinations.

Table. 3. Optical characteristics and statistical data of the regression equation

Parameters	UV Method	
	Zero order	**Area under curve**
λ_{max} (nm)	230 nm	225-235 nm
Beer's law limits (µg/ml)	6-16	6-16
Molar extinction coefficient (L mol^{-1} cm^{-1})	41.9281	30.1993
Sandell's sensitivity (µg/cm^2 -0.001 absorbance units)	0.007194	0.009917
Regression equation (Y^*)	Y = 0.142 X – 0.016	Y = 0.102 X – 0.0041
Slope (m)	0.0142	0.102
Intercept	-0.016	-0.009
Correlation coefficient (r^2)	0.999	0.999
LOD (µg/ml)	0.0422	0.206
LOQ (µg/ml)	0.1278	0.624

Y^* = m X + c where X is the concentration of clobazam in µg/ml and Y is the absorbance of the respective $\lambda_{max.}$

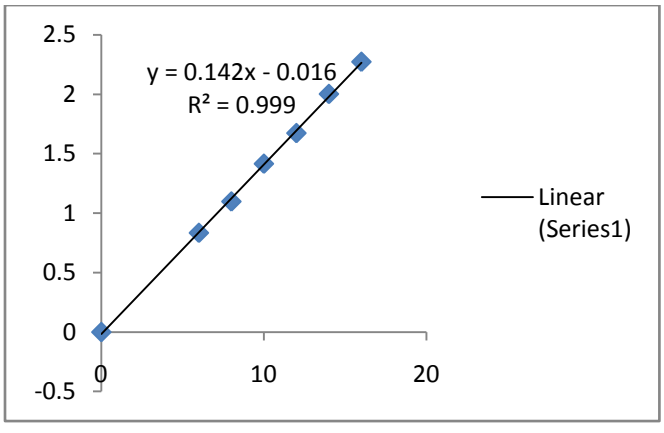

Figure 3. Calibration curve for Clobazam at 230 nm.

µg/ml of Clobazam. The calculated correlation coefficient (r) of least square linear regression was found to be 0.999 for the zero order and 0.999 for the area under curve method, respectively [9] (Figure 3 and 4).

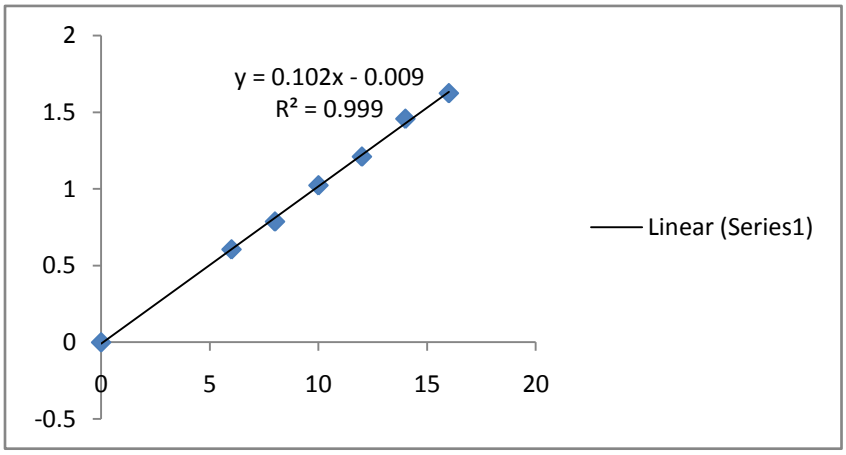

Figure 4.Calibration curve for Clobazam by area under curve spectroscopic method

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of standard bulk sample of Clobazam within the linearity range were taken and were added to the pre-analyzed formulation of concentration 6 µg/ml and percentage recovery values were calculated. They were found to be present within the range. The accuracy results were obtained for both zero order spectroscopy and area under curve method (Table 4 and 5).

Table. 4. Recovery study data of Clobazam by Zero order spectroscopy

Level of recovery	Amount of sample (µg/ml)	Amont of drug added (µg/ml)**	Amount of drug recovered (µg/ml)**	% Recovery ± S.D**
80%	6	4.8	4.69	97.90±0.274
100%	6	6	5.75	95.98±0.136
120%	6	7.2	7.13	99.10±0.158

** is average of six determinations

Table. 5. Recovery study data of Clobazam by Area under curve spectroscopy

Level of recovery	Amount of sample (µg/ml)	Amount of drug added (µg/ml)**	Amount of drug recovered (µg/ml)**	% Recovery ± S.D**
80%	6	4.8	4.64	96.85±0.3261
100%	6	6	5.77	96.31±0.5762
120%	6	7.2	6.95	96.63±0.4429

** is average of six determinations.

Precision

The precision of the proposed method was ascertained by determination of six replicates of same concentration of sample and standard for method precision and system precision. Both intraday precision and interday precision was carried out for zero order spectroscopy as well as area under curve method. The deviation between repeated readings was found to be present within the limit (Table 6 and 7).

Table. 6. Precision study data of Clobazam by Zero order spectroscopy

Concentration (µg/ml)	Intra-day absorbance mean ± SD**	% RSD	Inter-day absorbance mean ± SD**	% RSD
6	0.734 ±0.001789	0.243	0.847 ±0.000983	0.116
8	1.020 ±0.001033	0.101	1.108 ±0.010108	0.911
10	1.325 ±0.009649	0.727	1.418 ±0.001472	0.103
12	1.523 ±0.001414	0.092	1.686 ±0.000983	0.058
14	1.819 ±0.002137	0.117	2.010 ±0.00367	0.182
16	2.032 ±0.000753	0.037	2.285 ±0.006563	0.287

** is average of six determinations.

Table. 7. Precision study data of Clobazam by Zero order spectroscopy

Concentration (µg/ml)	Intra-day absorbance mean ± SD**	% RSD	Inter-day absorbance mean ± SD**	% RSD
6	0.527 ±0.001506	0.285	0.609 ±0.001862	0.305
8	0.739 ±0.001033	0.139	0.792 ±0.005514	0.696
10	0.960 ±0.004665	0.485	1.020 ±0.004676	0.458
12	1.105 ±0.003983	0.360	1.211 ±0.010852	0.895
14	1.311 ±0.009072	0.691	1.433 ±0.011153	0.778
16	1.455 ±0.009006	0.618	1.619 ±0.01642	1.014

** is average of six determinations.

Ruggedness

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. In the current study it was carried by two analysts for zero order spectroscopy as well as for area under curve method. The results thus obtained by two analysts were not having considerable deviation (Table 8 and 9).

Table. 8. Ruggedness study data of Clobazam by Zero order Spectroscopy

Sample	Label claim (mg)	Analyst 1		Analyst 2	
		Amount found (mg)	% Recovery ±SD**	Amount found (mg)	% Recovery ±SD**
Frisium	5	4.921508	98.43 ±0.035937	5.035211	100.70 ±0.09643
Cloba-5	5	5.0095	100.19 ±0.51754	4.8995	97.99 ±0.102909

** is average of six determinations.

Table. 9. Ruggedness study data of Clobazam by Area under curve Spectroscopy

Sample	Label claim (mg)	Analyst 1		Analyst 2	
		Amount found (mg)	% Recovery ±SD**	Amount found (mg)	% Recovery ±SD**
Frisium	5	4.782	95.65 ±0.5008	4.874	97.49 ±0.2420
Cloba-5	5	4.86	97.20 ±0.196334	4.991	99.82 ±0.415584

** is average of six determinations.

Limit of detection

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 6-16 µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

Limit of quantification

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve.

The method was validated according to International Conference of Harmonization guidelines for validation of analytical procedures. The proposed method showed absorption maxima at 230 nm and obeyed Beer's law in the concentration range of 6-16 µg/ml. The percentage recovery value indicates no interference from excipients used in formulation. The low value of percentage relative standard deviation shows that the developed method was precise. All statistical data prove validity of proposed method, which can be applied in industries for routine analysis of this drug from tablet.

CONCLUSION

For routine purposes it is always of interest to establish methods capable of analyzing a sample in a short period with due accuracy and precision. The main purpose of this study was to develop accurate, precise and economic methods for the determination of Cloabazam. In UV-Visible technique namely Zero-order Spectroscopic method, was applied without using any prior chemical pretreatment. The proposed UV spectrophotometric method is rapid, selective, simple, cost effective, fast and efficient. Finally, since there is only one pharmacopoeial method for determination of Clobazam in bulk and pharmaceutical formulations have been reported yet, the proposed method could be usefull and suitable for determination of Clobazam in bulk and pharmaceutical formulations.

ACKNOWLEDGEMENT

Authors wish to thank the Bharathi College of pharmacy, Bharathinagar for providing the facilities to carry out the research work.

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